Primitive quiescent CD34+ cells in chronic myeloid leukemia (CML) are relatively resistant to tyrosine kinase inhibitors, which may explain the persistence of detectable *BCR-ABL* transcripts following treatment with these agents. Conversely, allogeneic stem cell transplantation (SCT) can eradicate residual CML, suggesting that quiescent stem cells are eliminated by graft-versus-leukemia (GVL) effects. We studied the progeny of CD34+ cells after 4 days culture in serum-free media supplemented with interleukin-3, interleukin-6, stem cell factor, granulocyte-colony stimulating factor and Flt-3 ligand in 14 CML patients who subsequently received T cell depleted SCT from their HLA-identical sibling donors. Cycling CD34-negative and CD34+, and non-cycling quiescent CD34+ CML cells were isolated by fluorescence activated cell sorting. Fluorescence in situ hybridization in 7 representative CML patients revealed over 80% BCR-ABL positivity in both quiescent and cycling CD34+ and CD34-negative populations. The expression of *BCR-ABL* was the same in both cycling and quiescent CD34+ cell populations. Quiescent CD34+ cells from CML patients were lysed by natural killer (NK) cells from their donors, which had been expanded after 11–13 days culture with interleukin-2 and irradiated EBV-LCL. Quiescent CD34+ cells were less susceptible than their cycling CD34+ and CD34-negative counterparts to NK cytotoxicity. Compared to cycling populations, quiescent CD34+ cells from CML patients had higher expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors. Previous studies have demonstrated that bortezomib can sensitize malignant cells to NK-cell TRAIL (Lundqvist et al Cancer Res 2006). Bortezomib treatment of quiescent CD34+ CML cells enhanced their killing by expanded donor NK cells. This increased sensitivity to NK-cytotoxicity correlated with upregulation of TRAIL receptor DR4 on the surface of quiescent CD34+ CML cells. Bortezomib did not significantly affect the expression of MHC Class I, MIC A/B or Fas (CD95) on CD34+ quiescent or cycling cells. These results suggest that adoptive transfer of in vitro expanded donor NK cells with concomitant administration of bortezomib to the recipient may enhance cytotoxicity to quiescent CD34+ cells and may improve NK-mediated GVL effects. This may be particularly applicable to CML patients who are transplanted in more advanced stage disease, and are at a greater risk of relapse.

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### DISMAL RESPONSE TO HIGH DOSE METHYLPREDNISOLONE (MP) AFTER FAILURE TO RESPOND TO STANDARD DOSE IN PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) PATIENTS WITH ACUTE GRAFT-VERSUS-HOST-DISEASE (AGVHD)

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**Introduction:** Corticosteroids remain the primary therapy for aGVHD. The reported response rate to standard treatment (IV MP 2mg/kg/day for 14 days then tapered) is 70%. Patients who are refractory to standard treatment may be treated with high dose MP. We evaluated the response to high dose MP in children with aGVHD refractory to standard dose MP.

**Methods:** Children included in this evaluation underwent HSCT between June 1, 2002 and July 31, 2006; did not receive MP for aGVHD prophylaxis; developed ≥ grade II aGVHD; and received MP 2mg/kg/day as initial therapy. Response to aGVHD therapy, alternate aGVHD treatment, adverse effects attributed to MP and overall outcomes were documented.

**Results:** 50 children (29 male; mean age 8.9yrs (range 0.3–17yrs)) developed aGVHD during the study period. The median time to diagnosis of aGVHD ≥ grade II was 18 days (range 10–60 days). 34 children had single organ involvement alone; 36 (72%) responded to standard dose MP including 29 of 34 children with aGVHD involving a single organ. Of the 14 children who failed to respond to standard treatment, 12 children received high dose MP (≥20mg/kg/day) for 3 consecutive days followed by tapering dose. When high dose MP was started, 10 patients had skin, 8 gut, 7 liver and 1 lung aGVHD. 11 patients had ≥ grade III aGVHD when high dose MP was started. 10 patients received high dose MP at a median of 11 days (range 3–18days) after starting standard treatment while 2 patients received high dose MP after other non-steroid therapy failed. Only one patient with grade III aGVHD (stage 3 skin, stage 2 liver) had complete response. 2 patients had partial response but flared when MP was tapered. All patients who failed high dose MP needed further salvage therapy. All children who received MP developed hypertension and required antihypertensive medication. 16 children developed hyperglycemia (7 on high dose MP); 5 required insulin. While on high-dose or tapering of high-dose MP, 5 developed severe infection (2 fungal, 2 adenovirus, 1 bacterial). 22 out of the 36 patients who responded to standard treatment were available for follow-up; 13 (60%) are alive (median follow-up:39mos). 6 children (50%) who received high dose MP therapy died (median follow-up:46mos).

**Conclusions:** Children with aGVHD refractory to standard dose MP who subsequently receive high dose MP do not respond and experience significant adverse effects. Other salvage therapy should be considered in these patients.

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### *N-ACETYL-L*-CYSTEINE PROMOTES T CELL MEDIATED IMMUNITY IN ALLOGENEIC SETTINGS *IN VIVO* AND *IN VITRO*

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*N*-acetyl-*L*-cysteine (NAC) is a thiol antioxidant which stimulates glutathione synthesis in cells. Several studies indicate that NAC exerts effects on the immune system *in vitro*, although with conflicting results. Whereas some studies indicate that NAC has an inhibitory effect on T cell proliferation and activation of NF-κB, others suggest an activating effect. The ability of NAC to protect cells against oxidative stress by inducing glutathione has provided a rationale for using it to prevent veno-occlusive disease of the liver and liver toxicity after allogeneic stem cell transplantation (ASCT). Unfortunately, a prospective randomized study failed to show a positive effect of NAC on hepatotoxicity in ASCT patients. However, we observed that patients who received NAC as a therapy to treat liver toxicity (n = 73) had an increased prevalence of acute GvHD compared to patients who had been randomized to not receive NAC (n = 87) ($P$ = 0.04), indicating that NAC has an immunostimulatory effect *in vivo*. Importantly, the NAC-treated group only had an increased risk of grade II acute GvHD (30%) compared to the control group (10%), whereas severe acute GvHD grades III-IV in the groups were 8% vs 14%, respectively. These observations prompted us to thoroughly study the effect of NAC on T cell mediated immunity *in vitro*. We have found that low levels of NAC (0.4–3.2 mM) potentiate T cell proliferation and upregualtion of activation markers in response to alloantigens, whereas high concentrations (12.5–50 mM) are highly suppressive, which might explain the previously published conflicting data. Furthermore, T cells that have been stimulated in the presence of high concentrations of NAC (25 mM) in a primary mixed lymphocyte reaction (MLR) respond more vigorously when restimulated in a secondary MLR without NAC, compared to control secondary MLR which had been cultured without NAC during both stimulations. Preliminary data indicate that this observation partly can be explained by that NAC render T cells less apoptotic, and that T cells respond robustly when NAC has been metabolized. To summarize, we have found that NAC has an immunostimulatory effect on T cells under certain conditions, both *in vitro* and *in vivo*. These observations might be of clinical importance, as NAC potentially could be used as a therapeutic agent to promote T cell mediated immunity in immunocompromized ASCT patients with relapse of the underlying malignant disease.

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### INTRA-ARTERIAL STEROIDS FOR SYSTEMIC STEROID RESISTANT GRAFT-VS-HOST DISEASE

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Standard primary therapy for acute graft-vs.-host disease (GVHD) is systemic corticosteroids, but failure of systemic steroids to control acute GVHD is common and can result in very high non-relapse mortality. Overall, fewer than 30% of patients with systemic steroid resistant GVHD have complete or partial responses to available therapy, and 1 year survival remains 15% or less. There has been no generally accepted therapy for systemic steroid-refractory acute GVHD. Although little data on localized therapy for GVHD is published, intra-arterial injection of high-dose corticosteroids appears to be a viable option and sporadic publications have demonstrated several positive outcomes in patients receiving intra-arterial steroids. We report on 17 patients with systemic steroid resistant GVHD who have been treated with intra-arterial injections of high-dose corticosteroids at our institution in the past 2 years. A total of 23 treatments were performed with only three patients receiving more than one treatment. Sixteen of the patients received treatment for GI GVHD, of which three had concomitant hepatic involvement. One received treatment for hepatic GVHD only. The patient with isolated hepatic GVHD received treatments into the hepatic arteries and all patients with GI GVHD received treatments into the superior and inferior mesenteric arteries. Six of the patients (35%) demonstrated no response, and of those 3 died of GVHD and 3 died of other complications. Five patients (29%) showed partial response. Of these, 4 were discharged on TPN and oral meds 14 to 43 days after the intra-arterial treatment, the fifth patient became septic prior to discharge and expired. The remaining six patients (35%) showed complete response and were discharged on PO diet and oral meds 1 to 22 days after the intra-arterial treatment. No immediate treatment or procedure related complications were noted. These preliminary results reinforce that direct intra-arterial injection of methylprednisolone appears to be effective and safe in patients that have proven to be resistant to systemic steroids. In two patients, one with a complete and the other with a partial response, there was recurrence of GVHD. These patients raise the question of whether more than one initial treatment may be helpful in preventing recurrence. Larger, prospective studies need to be conducted to further evaluate this promising approach to systemic steroid refractory GVHD.

**Patient Characteristics, Response, and Current Status**

| Age/ Sex | Diagnosis | Location of Disease | Prior Treatment | Duration to Response | Number of Treatments | Response | Current Status (days post IAS) |
|---|---|---|---|---|---|---|---|
| 63/F | NHL | GI | IVS, INF | 14 days | 1 | PR | no GVHD (713) |
| 48/M | NHL | GI | IVS, ATG, INF x2, MSC | 21 days | 1 | PR | cGVHD (564) |
| 58/M | NHL | GI | IVS | 12 days | 1 | PR | D, ARDS, no GVHD (42) |
| 41/F | MM | GI | IVS, ATG | 43 days | 1 | PR | D, ARDS, no GVHD (53) |
| 50/F | AA | GI | IVS, ATG | 12 days | 1 | CR | cGVHD of Lungs, no GI GVHD (562) |
| 70/M | NHL | Hepatic | IVS, ATG | 22 days | 1 | CR | no GVHD (735) |
| 60/M | NHL | GI | IVS, INF | 1 day | 1 | CR | no GVHD (568) |
| 50/F | NHL | GI | IVS | 19 days p 1st, 7 days p 2nd | 2 | CR | no GVHD (700) |
| 71/M | NHL | GI | IVS, INF x4, ATG | | 5 | NR | D, GVHD (15) |
| 55/M | NHL | GI | IVS | 7 days | 1 | CR | D - perforation secondary to CMV colitis (404) |
| 39/F | NHL | GI | IVS | | 1 | NR | D, GVHD, CMV colitis (56) |
| 70/F | NHL | GI | IVS | | 1 | NR | D, GVHD, sepsis (50) |
| 38/M | NHL | GI | IVS | | 1 | NR | D, GVHD (15) |
| 60/F | NHL | GI | IVS, INF | 24 days | 1 | CR | D, recurrent GVHD (498) |
| 59/M | NHL | GI | IVS, INF | | 2 | NR | D, GVHD (11) |
| 61/F | MDS/ NHL | GI | IVS | 14 days | 1 | PR | D, sepsis, no GVHD (34) |
| 50/M | MDS/ NHL | GI | IVS, ATG | | 1 | NR | D, GVHD, sepsis (32) |

(*Continued*)

IAS indicates intra-arterial steroids; NHL, non hodgkin's lymphoma; MM, multiple myeloma; AA, aplastic anemia; MDS, myelodysplastic syndrome; GI, gastrointestinal; IVS, intra-venous steroids; ATG, anti-thymocyte globulin; INF, infliximab; MSC, mesenchymal stem cells; PR, partial response; CR, complete response; NR, no response; D, dead; GVHD, graft-vs-host disease; cGVHD, chronic GVHD; ARDS, acute respiratory distress syndrome; CMV, cytomegalovirus.

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### THERAPY OF STEROID-REFRACTORY ACUTE GvHD WITH CD52 ANTIBODY ALEMTUZUMAB IS EFFECTIVE AND SAFE

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Although in recent years techniques of allogeneic hematopoietic stem cell transplantation have improved considerably to allow the use of this procedure even for elderly and less fit patients, severe graft-versus-host disease (GvHD) remains the main obstacle to apply this therapeutic modality. Still, no standard therapy has been identified once a patient has developed severe acute GvHD and is refractory to high-dose steroids. The pre-emptive use of CD52 antibody alemtuzumab as part of the conditioning regimen may lead unnecessarily to relapse and increased infection rates. Commercially available alemtuzumab was used in 16 patients now evaluable, age range 13 to 68 years, with GvHD grade III and IV refractory to at least 7 days of high-dose steroids. Patients had undergone stem cell transplantation from family donors (n = 7) or matched unrelated donors (n = 9). Four had 1 or 2 antigen mismatch. All patients had severe gut and/or liver involvement. Initially, in three patients in rather critical situations start doses of alemtuzumab in the range of 70mg to 80mg (total) were applied in fractions over several days and repeated after 3 to 4 weeks. Impressive responses, such as bilirubin levels of 48mg/dl returning to normal within several weeks, were encouraging, but virus reactivation and bacterial infections were seen. This finding and considering the limited quantities of lymphoid tissue present early after transplantation requiring less antibody, the starting dose was reduced to 20 to 33 mg in the second, and 5–10mg in the third cohort, repeated every 2 to 3 weeks. CR, or near CR, were obtained in 11 of 13 patients of these two dosage groups. The longest follow-up is +38 months. Chronic GvHD was frequently observed. Pronounced lymphocyte depletion seems inevitable for efficacy, but infectious complications were limited. Thus, despite the need for close observation for infectious complications, alemtuzumab given sequentially in moderate doses has a substantial activity in severe steroid-refractory acute GvHD.

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### EXTRACORPOREAL PHOTOPHERESIS THERAPY FOR CHRONIC GRAFT-VERSUS-HOST DISEASE: RESPONSE IS ASSOCIATED WITH CONTENT OF DENDRITIC CELLS AND T-CELLS IN PERIPHERAL BLOOD AT INITIATION OF TREATMENT

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**Background:** Extracorporeal photopheresis (ECP) is used to treat cGVHD, but its mechanism has not been fully defined. One model for the mechanism of ECP in cGVHD is dendritic cell (DC) depletion and T-cell modification. We tested this hypothesis by determining the numbers of circulating DCs and T-cells prior to ECP and